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Plasma triglyceride metabolism

D. S. ROBINSON

From the Department of Biochemistry, University of Leeds

Knowledge of the way in which triglyceride is transported in the blood and of the factors that influence the transport process is essential for a proper understanding of the overall distribution of lipids in the plasma that is observed in health and disease. The following account deals with triglyceride transport in normal individuals and provides a basis for comparison with states of abnormal lipid metabolism.

Because of their water insolubility, triglycerides are transported in the plasma in combination with other more polar lipids (phospholipids) and proteins, as well as with cholesterol and cholesterol esters, in complex macromolecules known as lipoproteins. The structure of the lipoproteins does not depend on covalent linkages between the various components but is determined rather by a combination of polar and non-polar interactions between them. It seems likely that the essentially non-polar triglyceride (and cholesterol ester) is largely in the centre of the lipoprotein, with the more polar protein and phospholipid components at the surface, their polar groups being directed outwards so as to stabilize the whole structure in the aqueous plasma environment.

The concentration of lipoprotein triglyceride in the plasma at any given time must represent a balance between the rate of entry into the plasma and the rate of removal. A change in concentration may therefore be the result of a change in either or both of these factors. Moreover, a primary change in one may result in a secondary change in the other. Thus, perhaps the main question to be asked, in any situation where the plasma triglyceride concentration is abnormally high, is whether this is due to a rise in the rate of entry or to a fall in the rate of removal.

Entry of Triglyceride into the Plasma

FROM THE INTESTINE

About 30 to 40% of our calorie intake is normally in the form of fatty acids contained in the dietary triglyceride. Digestion in the intestinal lumen breaks down this triglyceride into free fatty acids and monoglycerides, and these are absorbed by the intestinal cells and re-synthesized into triglyceride which is then released into the lymphatics in lipoproteins called chylomicrons. These contain about 90% of triglyceride, 5% of cholesterol (mainly as the ester), 5% of phospholipid, and a very small amount (less than 0·5%) of protein. Although the amount of protein is so small, there is a good deal of evidence that its presence is necessary for the release of the chylomicrons. For example, in abetalipoproteinaemia, a genetically determined deficiency disease in which this protein cannot be made in the body, triglyceride is not released from the intestinal cells.

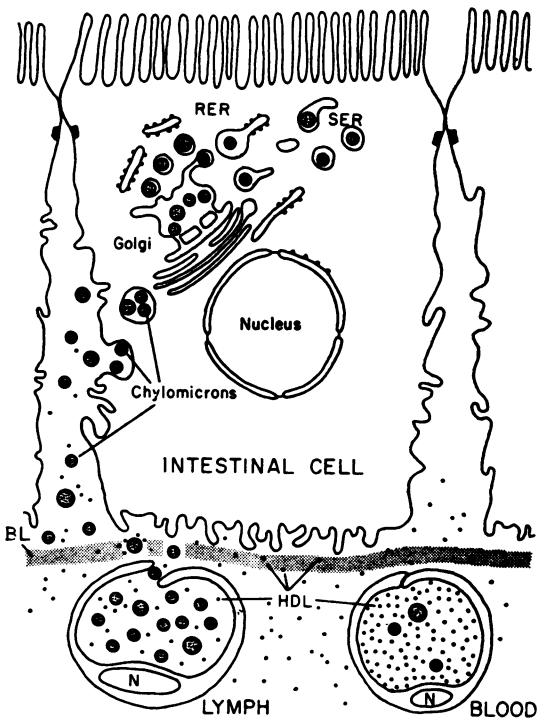

Fig 1 *The plasma lipoprotein secretory mechanism in the intestine (from Hamilton, 1972).* RER = *rough endoplasmic reticulum,* SER = *smooth endoplasmic reticulum,* HDL = *high-density lipoprotein,* BL = *basal lamina.*
'Lymph' and 'blood' depict respectively a lymphatic and a blood capillary. (Reproduced from the article by R. L. Hamilton in *Advanc. exp. Med. Biol.*, **26**, 10, by kind permission of the Editors and Publishers, Plenum Press, New York.)

The details of the processes involved in chylomicron formation and release are still obscure, and fig 1 merely provides a diagrammatic representation of what takes place in general terms. The chylomicrons are probably formed in the cisternae of the rough and smooth endoplasmic reticulum and the Golgi apparatus of the intestinal cell, whence they are transported to the lateral surfaces and released into the interstitial spaces. From these they enter the lymphatics and are conveyed to the bloodstream via the thoracic duct.

An increased influx of chylomicron triglyceride into the plasma follows the ingestion of each meal and this persists for the several hours during which fat is absorbed. It causes an increase in plasma triglyceride concentration and, because the chylomicrons are large enough to scatter light (up to 0·5 μ in diameter), the plasma becomes turbid or lipaemic so as to produce what is commonly called an alimentary lipaemic response. However, this rise in plasma triglyceride concentration is relatively small when related to the magnitude of the increased influx into the plasma, because the rate of removal of the chylomicron triglyceride also rises rapidly to approximate to the increased rate of entry.

Even in starvation, or in individuals on diets containing little fat, some triglyceride is released from the intestinal cells into the lymphatics, though the amount is naturally much reduced. In such circumstances the lipoprotein complexes contain less triglyceride and are therefore smaller in size and more dense than the chylomicrons. However, it is significant that they contain the same non-triglyceride components and it seems best to picture lipoproteins released from the intestinal cells as really having no fixed size. Thus, when the amount of triglyceride released from the intestinal cell is small, the complexes themselves are relatively small; on the other hand, when the amount released is large, as during the absorption of a meal containing fat, the complexes increase in size by acquiring a greater load of triglyceride until they become chylomicrons as these are commonly defined. There may also be an increase in the number of chylomicrons released when a great deal of triglyceride has to be transported from the intestinal cells, but it nevertheless seems likely that much of the rise in output is achieved simply by an increase in the triglyceride loading of each chylomicron unit.

FROM THE LIVER

The liver is the second site of triglyceride release into the plasma. The source of the fatty acids present in the triglyceride entering the blood from this organ depends markedly on the nutritional state. Thus, in a fasting individual, fatty acids are mobilized from the adipose tissue stores and are transported in the plasma in unesterified form bound to the plasma albumin. Most are carried directly to tissues such as muscle and are oxidized. However, some—about 30 to 40% in the resting state—enter the liver and here a proportion is directly oxidized, either completely to carbon dioxide and water or partially to ketone bodies, while a proportion is re-esterified within the liver and released again into the plasma as triglyceride. In this state therefore the fatty acids of the plasma triglyceride are derived indirectly from the fatty acids mobilized from adipose tissue.

Contrast this with the situation immediately after the ingestion of a meal by an individual on a low-fat, high-carbohydrate diet. Here part of the dietary carbohydrate is converted to fatty acid in the process known as lipogenesis. This conversion occurs both in adipose tissue, in which case the fatty acid is directly esterified and stored in that tissue as triglyceride, and in the liver. In the latter, the fatty acid is also esterified to yield triglyceride but it is then released into the plasma in lipoproteins. Thus, in this situation the fatty acid moiety of the plasma triglyceride has come mainly from the dietary carbohydrate.

Despite these variations in the source of the triglyceride fatty acids released by the liver, it is important to realize that the release process itself is a continuous one and that, except during the absorption of dietary fat, the liver is the main contributor of triglyceride to the plasma. Moreover, though the diurnal variations in the rate of triglyceride release from the liver are normally considerably less than those from the intestine, long-term variations in hepatic release rate probably can occur, in particular as a result of changes in dietary or hormonal status. This raises the question as to how far such changes could be responsible *per se* for similar long-term alterations in plasma triglyceride concentration and for differences in triglyceride concentration between populations. This has proved a controversial topic but it is perhaps worth mentioning that there are certainly some situations where alterations in plasma triglyceride concentration are due to changes in the rate of release of hepatic triglyceride. For example, a block in hepatic triglyceride release is produced by a number of liver poisons such as carbon tetrachloride and white phosphorus. This is undoubtedly the primary cause of the very low plasma triglyceride concentration and the marked accumulation of triglyceride in the liver—the 'fatty liver'—found in poisoning with these substances.

Our knowledge of the process of triglyceride release from the liver, much of it derived from studies on the perfused liver *in vitro*, suggests that it is very similar to triglyceride release from the intestine.

Moreover, triglyceride release from the liver is prevented in the same genetic disorder (abeta-lipoproteinaemia) in which release from the intestine is blocked. As stated above, this disorder is characterized by an inability to synthesize a protein species which normally forms part of the lymph chylomicrons, and this same protein is also a normal constituent of the triglyceride-rich lipoproteins released by the liver. Again, as in the intestine, the size of the lipoprotein complexes formed by the liver varies according to the amount of triglyceride that is being released. Thus, high rates of release result in large complexes with a high triglyceride load and a correspondingly low density. In fact, the lipoprotein complexes released from the liver under such conditions may reach a size not much below that of the chylomicrons, even though they normally have a somewhat lower triglyceride content and, therefore, a higher density.

Transport of Triglyceride by Lipoproteins in the Plasma

Some of the lipoproteins present in the plasma are essentially those which have already been described as being produced by the intestine and the liver. In the starved individual or the individual on a low-fat, high-carbohydrate diet, for example, we find lipoproteins similar in their size and their high triglyceride content to those released by the liver. These are known either as the 'very-low-density lipoproteins' (VLDL) because they are the plasma lipoprotein fraction most readily separated by flotation in the ultracentrifuge, or as 'pre-β-lipoproteins' on the basis of their electrophoretic behaviour. During the absorption of dietary fat there are also present chylomicrons which because they are of still lower density than the VLDL, are even more readily separated by flotation in the ultracentrifuge.

Though most of the plasma triglyceride is present in one of these two fractions, the plasma also contains other lipoproteins. These, which carry most of the plasma cholesterol and phospholipid, are again generally classified according to their ultracentrifugal or electrophoretic behaviour. It is usual to distinguish two main groups which are smaller and, because of their lower triglyceride content, have a higher density than the chylomicrons and the very-low-density lipoproteins. They are called either 'low-density lipoproteins' (LDL) or 'high-density lipoproteins' (HDL) on the basis of their relative densities and lipid/protein ratios, and they have respectively β and α electrophoretic mobility.

Recent work has shown that this relatively simple classification into four main groups of lipoproteins is far from adequate. Nevertheless it will suffice for our present purposes in that it now seems very probable that the LDL and at least part of the HDL in the plasma originate from the VLDL and the chylomicrons. The plasma VLDL, for example, contain several distinct protein species, one of which is the same as that of the LDL, while the others are present in the high-density lipoprotein. It seems likely, therefore, that removal of the triglyceride moiety of the VLDL from the plasma (by mechanisms described below) leaves a residual triglyceride-poor complex which then breaks up in the plasma to give the low- and high-density lipoproteins. Moreover, though the picture with respect to the chylomicrons is less thoroughly studied, it is probable that here too the same protein components are present as in the VLDL and that again the process of triglyceride removal from the plasma leaves a triglyceride-poor complex which then fragments into low- and high-density lipoproteins.

The chylomicrons and VLDL already contain the protein moiety (apoprotein) that they have in common with the LDL at the time when they are released from the intestine or the liver. Indeed, in abeta-lipoproteinaemia it is the failure to synthesize this apoprotein which is the cause of the block in triglyceride release. However, they do not appear at this stage to contain all their HDL apoproteins. It seems, in fact, that much of the association of the HDL apoprotein with chylomicrons and VLDL occurs after the entry of these into the bloodstream. Moreover, whereas the formation in the plasma of LDL from the VLDL and chylomicrons through the removal of triglyceride from the plasma is irreversible, in that the LDL apoprotein is not re-utilized, this is probably not so for the HDL protein species. Some of the latter, for example, exchanges freely with HDL proteins in the very low-density lipoprotein. It seems likely therefore that once the protein components of the HDL are released after triglyceride removal, they are able to re-associate with new chylomicrons and VLDL entering the plasma from the intestine and the liver.

Removal of Triglyceride from the Plasma

Since the rate of removal of triglyceride from the bloodstream is normally adjusted quickly to balance its rate of entry, then whenever the latter varies, the former will also change. Thus, when the rate of entry of chylomicron triglyceride increases during the absorption of dietary fat, the rate of removal also increases. Indeed, in experimental animals, the circulating half-life of the chylomicron triglyceride fatty acid under such conditions is only a few minutes.

This rapid removal has to be reconciled with the

fact that the lipoprotein complexes, of which the triglyceride forms a part, are so large that their passage as such across the blood vessel walls in most of the body tissues could only be extremely slow. The rapid removal of the chylomicron (and VLDL) triglyceride fatty acid must, therefore, entail the operation of a process acting selectively on the triglyceride component.

It is now recognized that this selective process involves the hydrolysis of the triglyceride moiety by a lipase called clearing factor or lipoprotein lipase. Present evidence suggests, moreover, that the site of action of this enzyme on the VLDL and chylomicron triglyceride is at the luminal surface of the capillary endothelium of the extrahepatic tissues, and that for this purpose the VLDL and chylomicrons have to be trapped or sequestered at this site as they pass through the capillary lumen. This sequestration is of particular interest because it requires an association to take place at the endothelial cell surface between the enzyme and the chylomicrons or VLDL in the bloodstream. Such an association can readily be shown *in vitro* using enzyme extracted from the tissues, and seems to depend on the presence in the chylomicrons and the VLDL of one or more of the particular apoproteins which they hold in common with the HDL. In fact, it appears that the association of HDL apoprotein with VLDL and chylomicrons, which occurs mainly after these have been released into the plasma, is a prerequisite for the hydrolysis of the triglyceride by clearing factor lipase at the endothelial cell surface and hence for the removal of the triglyceride fatty acids from the bloodstream.

The fatty acids released by hydrolysis at the endothelial cell surface are then in a situation where they can readily pass out of the bloodstream into the tissues (fig 2). The free passage of unesterified fatty acids across cell membranes is well recognized. In fact, much of the fatty acid mobilized from the triglyceride stored in adipose tissue in times of calorie deficit is taken into the cells from the bloodstream in this way. However, there is one important difference between the uptake by the tissues of the unesterified fatty acids released from adipose tissue and of those produced by hydrolysis of the chylomicron and VLDL triglyceride. Whereas unesterified fatty acids carried in the bloodstream enter the different body tissues in accordance with the blood flow through those tissues, the uptake of triglyceride fatty acids by a particular tissue depends on the activity of clearing factor lipase in that tissue's capillaries.

The action of clearing factor lipase at the endothelial cell surface therefore not only facilitates the removal of triglyceride fatty acid from the blood but

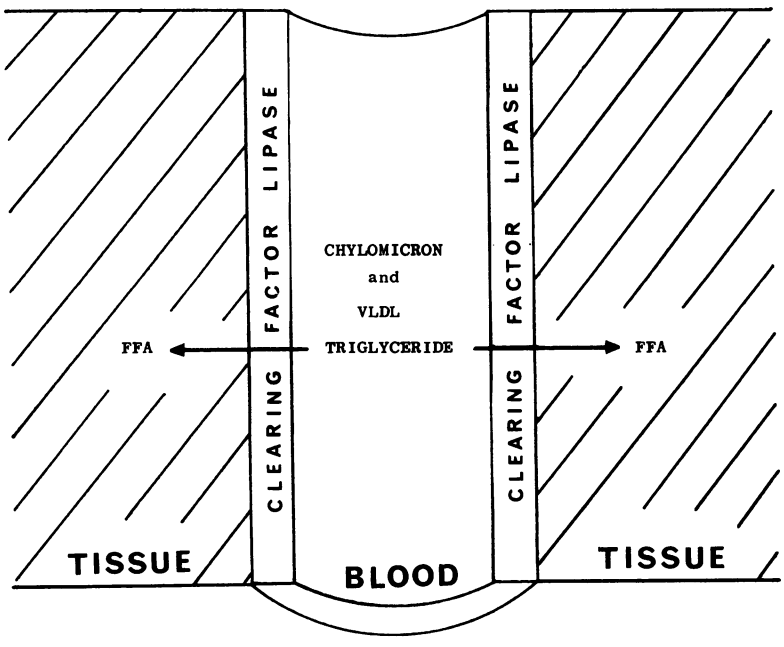

Fig 2 *The site of action of clearing factor lipase on chylomicron and VLDL triglyceride. FFA=free or unesterified fatty acids.*

also determines its location, and this has important consequences. For example, in a state of calorie excess, that proportion of the triglyceride fatty acid in the bloodstream that is in excess of the immediate caloric needs is taken up by adipose tissue, most of the fatty acids being reconverted to triglyceride therein and stored, while the remainder of the uptake in such a situation occurs predominantly in muscle where most of the fatty acid is probably oxidized directly. In contrast, in a state of calorie deficit as during fasting, the tissues derive their energy primarily from the oxidation of unesterified fatty acids carried to them in the blood after their mobilization from adipose tissue. There is still triglyceride in the blood in VLDL in those circumstances but this, instead of being taken up by adipose tissue for storage, is now directed away from this tissue and towards muscle to supplement the supply of energy from the mobilized fatty acids. It now seems clear that this switch in triglyceride fatty acid uptake is achieved through changes in the activity of clearing factor lipase in the tissues concerned. Thus, fasting results in a fall in the activity of the enzyme in adipose tissue and an increase in its activity in muscle. A similar example is provided by the mammary gland during lactation. Here there is a dramatic increase in clearing factor lipase activity and uptake of triglyceride fatty acid from the plasma at parturition, when triglyceride fatty acids are required for secretion of milk triglycerides, and this persists until weaning when both uptake and enzyme activity fall together.

If clearing factor lipase determines the pattern of triglyceride fatty acid removal from the blood in this way, what regulates these tissue-specific changes in activity? While no complete answer to this question can be given at the present time, it appears likely that, at least with respect to the enzyme in adipose tissue, the control is hormonal. Thus, it has been shown in various *in-vitro* systems that insulin favours an increase in the activity of the enzyme in such tissue while other hormones, eg, the catecholamines, ACTH, and glucagon, inhibit the rise that is promoted by insulin.

These hormones all have an opposite effect on the quite different lipase present in adipose tissue which is concerned with the mobilization of the fatty acids that are stored there as triglyceride and which is most active in the tissue when clearing factor lipase is least active. There is good evidence that the activity of this mobilizing lipase increases in the tissue as the fat cell cyclic AMP concentration increases, and that hormonal effects on it are normally mediated through changes in this cyclic AMP concentration. In fact, there are two forms of mobilizing lipase, and cyclic AMP promotes the conversion of one form to another with higher activity. It is of particular interest in the present context, therefore, that there is also evidence that a rise in the cyclic AMP concentration in the fat cell can, either directly or indirectly, lower the clearing factor lipase activity of adipose tissue as it raises that of the mobilizing lipase, and that clearing factor lipase too may exist in more than one form. Thus, changes in cyclic AMP concentrations in adipose tissue, brought about by hormones, could simultaneously have opposite effects on the uptake and storage of plasma triglyceride fatty acid by the tissue and on the mobilization of fatty acid from the existing stored triglyceride.

There is much more to be learnt about the hormonal control of clearing factor lipase activity. For example, it is not yet clear how a particular hormone may alter the activity of the enzyme in adipose tissue in one direction and that of the enzyme in muscle in the opposite one. However, we are at least beginning to understand how changes in nutritional and hormonal balance may affect the activity of the enzyme in particular tissues, and hence the pattern of triglyceride fatty acid removal from the blood. Moreover, once it is appreciated that the action of the enzyme is under hormonal control, it is possible to envisage how differences in its activity, and hence in the total capacity for triglyceride removal from the blood, may exist in different individuals.

One further feature of the enzyme finally remains to be considered. Reference has already been made to evidence which suggests that it acts at the capillary endothelial cell surface. It is now clear, however, that not all the enzyme present in a given tissue is localized at this particular site and this applies to both muscle and adipose tissue; in the latter, for example, it has been shown quite clearly that some enzyme is located inside the fat cells. Moreover, while it seems that it is only the enzyme at the endothelial cell surface which is functional with respect to triglyceride fatty acid uptake, there is also evidence that the enzyme which is non-functional in this respect may be the precursor of the functional enzyme at the endothelial cell surface. If this is so, then clearly the enzyme has to be transported from the fat cell to the endothelial cell surface in order to exert its activity and, in adipose tissue for instance, regulation of the enzyme's activity by hormones could be exerted either in the fat cell, or during transport of the enzyme from the cell, or at the endothelial cell surface (fig 3).

This point is of some importance because the activity of functional clearing factor lipase in the tissues may be reduced in some hypertriglyceridaemic conditions, and this may well be the prime cause of

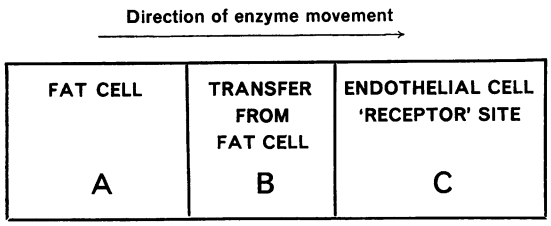

Fig 3 *Possible sites for hormonal control of adipose tissue clearing factor lipase (after the figure from Robinson and Wing, 1970).* A *Site of synthesis of proposed 'precursor' form;* A or B *possible sites of conversion to 'functional' form;* C *functional site.*

that hypertriglyceridaemia. However, such a deficiency may be due either to a lack of total enzyme in the tissue, or to a deficiency in the conversion of the non-functional to the functional enzyme. Such considerations as the foregoing may have particular implications for clinical studies on hypertriglyceridaemia in man. For example, at the present time the most convenient assay of clearing factor lipase in man involves the measurement of the activity of the enzyme as it appears in the plasma after the intravenous injection of heparin. This release of the enzyme into the bloodstream by heparin has not been discussed here because it tends to obscure a proper appreciation of the normal functioning of the enzyme in the tissues. However although such postheparin plasma activity may give some measure of the total activity of the functional enzyme in an individual's body tissues—in so far as it probably represents enzyme that is released by heparin from the endothelial cell surface—it can give no measure of the total activity in particular tissues, nor can it indicate the capacity to renew the supply of functional enzyme.

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